Synthesis and Leishmanicidal Evaluation of Novel 4-Substituted-2,2-Dimethyl-7-(prop-2-ynyloxy)Chromenes

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Substituted 4-chromenes were synthesized via the Grignard coupling reaction of various substituted phenyl- and benzylmagnesium chlorides with 2,2-dimethyl-7-(prop-2-ynyloxy)chroman-4-one (1) in relatively good yield (60%-85%). The benzylic-substituted derivatives were a mixture of 2 exo and endo regioisomers, and the exo compounds were a mixture of E and Z isomers. Structural analysis of the exo and endo isomers, as well as E and Z of exo isomers were based on 1H-NMR, 13C-NMR, and NOESY experiments. The synthetic compounds were examined for in vitro activity against Leishmania major and compound 9 (a, b) was the most active, with inhibitory activity of 36%.

Key Words: Synthesis, chromene, leishmanicidal activity.

Introduction

More than 17 species of Leishmania are known to infect humans. Unfortunately, in practice there are no effective vaccines against the parasite. Thus, control of the disease relies primarily on chemotherapy. The

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species have been characterized on the basis of biochemical and molecular differences, and these differences provide an explanation for variation in clinical response and species sensitivity to the pentavalent antimonial; sodium stibogluconate and meglumine antimonate (glucantime), used for the treatment of leishmaniasis over the past 50 years. Generally, the antileishmanial drugs on the market possess severe side effects, are expensive, require long-term use, and do not provide complete eradication of the disease. In addition, resistance to current drugs develops rapidly and large-scale resistance to pentavalent antimonials has been reported in some parts of the world (for example in India and Sudan), leading to their obsolescence.\(^1\)–\(^3\) Moreover, the biological potency of heterocyclic chromenes has been widely documented and considerable effort has been made to explore new routes for the synthesis of therapeutically more efficient heterocyclic chromenes. In order to find new drugs with more potent activity against the *Leishmania* parasite, we have engaged in a program to investigate some new synthetic compounds.\(^4\)–\(^8\) In our previous paper we reported the synthesis and potential antileishmanial activity of 4-substituted-2,2,8,8-tetramethylpyrano[2,3-f] chromene A (Figure 1). Herein we report the synthesis and leishmanicidal activity of 4-6 and 8-15.

![Figure 1. Structure of the synthetic leishmanicidal chromenes.](image)

Results and Discussion

Compound 2 (Scheme) was prepared via the reaction of propargyl bromide and 2,2-dimethyl-4-chromone 1 in acetone in the presence of K\(_2\)CO\(_3\). Compounds 4-6 and 8 (a, b)-15 (a, b) were synthesized via the reaction of compound 2 with appropriate Grignard reagents and subsequent acidic dehydration reaction in refluxing 1 N HCl (Scheme).\(^9\)–\(^11\) Compounds 8 (a, b)-15 (a, b) were a mixture of 2 regioisomers and their structures were established with \(^1\)H-NMR analysis. In addition, exo isomers (9a-15a) were a mixture of \(E\) and \(Z\) isomers. It should be noted that none of these isomers could be separated by conventional methods.\(^12\),\(^13\) Differentiation between exo and endo isomers, as well as between \(E\) and \(Z\) of exo isomers was based on \(^1\)H-NMR, \(^13\)C-NMR, and NOESY experiments. With \(^1\)H-NMR, observation of a doublet at 2.54-2.72 ppm (3-CH\(_2\) of compound 8a-15a) and a singlet at 3.64-3.95 ppm (benzylic CH\(_2\) of compound 8b-15b) showed the existence of both exo and endo isomers, respectively, in ratios of 27:73-77:23. Moreover, the exo product appeared to be a mixture of \(E\) and \(Z\) stereoisomers, which were assigned based on the anisotropic effect of the substituted phenyl ring on H\(_5\) of the \(Z\) isomer. In the \(E\) isomer, H\(_5\) of compound 9a appeared as a doublet at 7.60 (\(J = 8.8\) Hz) and in the \(Z\) isomer the H\(_5\) appeared at 7.55 ppm (\(J = 8.8\) Hz); however, in most compounds the \(Z\) isomers were a minor and undetectable product. The configuration of the \(E\) isomer as a major product was confirmed with
2D NOESY NMR spectroscopy and the assignment of stereochemistry of compound 11a was consistent with the observed strong cross peak between H₅ and vinylic-H (Ar-CH = C), and the relatively weak cross peak between H₃ and the ortho-H phenyl ring. In addition, based on ¹³C-NMR the 3-CH₂ of the endo isomers appeared as 2 separate signals at 32.7-37.2 and 37.1-37.4, respectively. The vinylic CH (exo isomer) and olefinic CH (endo 3-CH) appeared as 2 separate singlets at 118.7-123.3 and 124.1-125.5, respectively. Dept-135 and Dept-90 experiments confirmed the CH₂ and CH groups.

In evaluating inhibitory activity against *L. major* (Figures 2 and 3) 3 compounds (5, 8, and 9) proved to be effective after 72 h of incubation, with growth inhibition > 20% (Table). The target compound 8, having no substitution in the phenyl ring, had inhibitory activity > 30% and the most active compound was chromene 9.

![Scheme](image)

**Experimental**

All chemicals and reagents were obtained from Merck Chemical Company (Darmstadt, Germany) and Sigma-Aldrich Chemical Company (Steinhein, Germany). ¹H-NMR spectra were measured using a Bruker 500 MHz spectrometer (Brucker, Rheinstetten, Germany) and chemical shifts are expressed as δ (ppm), with
tetramethylsilane as the internal standard. IR spectra were taken using a Nicolet FT-IR Magna 550 spectrograph (KBr disks) (Nicolet, Madison, WI, USA). MS spectra were obtained with a Finnigan MAT TSQ-70 spectrometer (Finnigan Mat, Bremen, Germany). The purity of each compound was confirmed by TLC using different mobile phases. The results of elemental analyses (C, and H) were within ± 0.4% of the theoretical values for C, and H.

Figure 2. *L. major* growth curve. Promastigotes were seeded at approximately $2 \times 10^6$ cells/mL, and during 8 days cell concentration was determined daily using a Neubauer’s chamber. (●) Growth phases of untreated cells. (♦) Promastigote growth rate after treatment with glucantime as a standard. Each time point represents the mean ± SD of 3 replicates.

Figure 3. The effects of the synthetic agents on the in vitro growth rate of *L. major* promastigotes. The parasites were cultured for 24 h (grey bar) and 72 h (black bar) in the presence of the compounds as described in the material and methods section. The results are given as percentages of inhibition (mean ± SD).

**2,2-Dimethyl-7-(prop-2-ynyloxy)chroman-4-one (2)**

To a solution of 1 (1.06 g, 5.52 mmol) in dry acetone (30 mL) and K$_2$CO$_3$ (0.92 g, 6.07 mmol) under argon was added propargyl bromide (1.6 mL, 6.07 mmol). The solution was allowed to stir at room temperature for 24 h, and then the precipitate was filtered and washed with acetone. The collected acetone was concentrated at reduced pressure to give a crude oil, and the residue was extracted with EtOAc ($3 \times 50$ mL) and water. The combined organic phase was washed with 1N HCl ($3 \times 50$ mL), saturated NaHCO$_3$, and brine. After
Table. Comparison of the inhibitory effect of the compounds on the viability of *L. major* promastigotes under in vitro conditions.

<table>
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<tr>
<th>entry</th>
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<th>Inhibitory % 72 h</th>
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<tr>
<td>2</td>
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<tr>
<td>4</td>
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<td>15</td>
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</tr>
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<td>A (Figure 1)</td>
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<tr>
<td>Glucantime</td>
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drying (Na$_2$SO$_4$) the solvent was removed under reduced pressure to give a residue, which was purified with column chromatography (silicagel, 30 g) eluted by hexane/ethyl acetate (4:1) to provide compound 2 (1.25 g, 4.99 mmol, 90%) as pale red crystals (hexane/EtOAc, 4:1). mp: 118-120 °C; IR (film): $\nu$ 3282 (HC$\equiv$C), 2110 cm$^{-1}$ (C$\equiv$C); $^1$H-NMR (CDCl$_3$): $\delta$ = 1.45 (s, 6H, 2-CH$_3$), 2.56 (t, 1H, $J$ = 2.4 Hz, HC$\equiv$C), 2.65 (s, 2H, 3-CH$_2$), 4.69 (d, 2H, $J$ = 2.4 Hz, OCH$_2$), 5.28 (s, 1H, 3-CH, enol form), 6.45 (d, 1H, $J$ = 2.4 Hz, H$_8$), 6.59 (dd, 1H, $J$ = 2.4 Hz, $J$ = 8.8 Hz, H$_9$), and 7.80 (d, 1H, $J$ = 8.8 Hz, H$_5$); $^{13}$C-NMR (CDCl$_3$): $\delta$ = 26.7 (q), 48.5 (t), 55.9 (t), 76.3 (s), 79.7 (d), 102.4 (d), 109.6 (d), 114.6 (s), 128.4 (d), 161.9 (s), 164.1 (s), and 191.6 (s); MS $m/z$ (%): 230 (M$^+$, 63), 229 (69), 214 (77), 187 (14), 175 (51), 145 (85), 117 (100), 108 (37), 101 (79), 91 (35), 63 (42), and 50 (69); anal. calcd. for C$_{14}$H$_{14}$O$_3$: C, 73.03; H, 6.13. Found: C, 73.19; H, 6.29.

**2,2-Dimethyl-4-phenyl-7-(prop-2-ynyloxy)-2H-chromene (4)**

To a stirred solution of 2 (0.3 g, 1.3 mmol) in dry ether (3 mL) was added phenylmagnesium bromide (2.5 mL, 2 mmol, 0.8 M) under argon. The resulting mixture was stirred and refluxed for 18 h. After cooling the organic phase was washed with 1N HCl (3 × 5 mL). The solvent was evaporated under reduce pressure to give an oil.
To this oily residue was added 2N HCl (15 mL), which was refluxed for 12 h. After cooling it was extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with 1N NaOH and saturated aqueous NaHCO₃, and then was dried (Na₂SO₄). After filtration the solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (silica gel = 25 g, hexane/EtOAc = 5:1) to give 4 (0.32 g, 1.1 mmol, 85%) as a colorless oil.

IR (film): ν 3282 (HC≡C), 2110 cm⁻¹ (C≡C); ¹H-NMR (CDCl₃): δ = 1.48 (s, 6H, 2-CH₃), 2.53 (t, 1H, J = 2.4 Hz, HC≡C), 4.66 (d, 2H, J = 2.7 Hz, OCH₂), 5.47 (s, 1H, 3-CH), 6.46 (dd, 1H, J = 2.4 Hz, J = 8.5 Hz, H₆, 6.54 (d, 1H, J = 2.4 Hz, H₈), 6.93 (d, 1H, J = 8.5 Hz, H₅), and 7.32-7.38 (m, 5H, Ar); ¹³C-NMR (CDCl₃): δ = 27.6 (q), 50.2 (t), 75.6 (s), 76.3 (s), 78.4 (d), 103.4 (d), 107.5 (d), 116.4 (s), 125.3 (d), 125.9 (d), 126.3 (d), 126.7 (d), 128.2 (d), 128.6 (d), 134.4 (s), 138.5 (s), 154.6 (s), and 158.5 (s); MS m/z (%): 290 (M⁺, 100), 275 (98), 272 (51), 236 (59), 208 (91), 199 (29), 178 (42), 165 (51), 152 (27), 117 (27), 105 (29), 91 (30), and 77 (45); nal. calcd. for C₂₀H₁₃ClO₂: C, 73.96; H, 5.28. Found: C, 73.80; H, 5.35.

Other compounds, 5, 6, and 8 (a, b)-15 (a, b), were prepared similarly.

4-(4-Chloro-phenyl)-2,2-dimethyl-7-(prop-2-ynyloxy)-2H-chromene (5)

A colorless oil (75%); R (film): ν 3289 (HC≡C), 2110 cm⁻¹ (C≡C); ¹H-NMR (CDCl₃): δ = 1.48 (s, 6H, 2-CH₃), 2.53 (t, 1H, J = 2.4 Hz, HC≡C), 4.72 (d, 2H, J = 2.7 Hz, OCH₂), 5.47 (s, 1H, 3-CH), 6.46 (dd, 1H, J = 2.6 Hz, J = 8.5 Hz, H₆, 6.52 (d, 1H, J = 2.6 Hz, H₈), 6.87 (d, 1H, J = 8.5 Hz, H₅), 7.27 (d, 2H, J = 8.3 Hz, H₃, 5-4-ClPh), and 7.36 (d, 2H, J = 8.3 Hz, H₂, 6-4-ClPh); ¹³C-NMR (CDCl₃): δ = 27.6 (q), 55.8 (t), 75.6 (s), 76.3 (s), 78.4 (d), 102.1 (d), 107.1 (d), 116.0 (s), 119.9 (d), 126.0 (d), 128.4 (d), 130.0 (d), 131.0 (s), 133.4 (s), 136.9 (s), 158.6 (s), and 165.8 (s); MS m/z (%): 326 (M⁺ + 2, 10), 325 (M⁺ + 1, 6), 324 (M⁺, 30), 310 (28), 274 (15), 228 (28), 213 (100), 173 (64), 161 (25), 146 (24), 106 (27), 92 (48), 89 (65), and 63 (78); nal. calcd. for C₂₀H₁₃ClO₂: C, 73.96; H, 5.28. Found: C, 73.80; H, 5.35.

4-(4-Methyl-phenyl)-2,2-dimethyl-7-(prop-2-ynyloxy)-2H-chromene (6)

A colorless oil (60%); IR (film): ν 3295 (HC≡C), 2115 cm⁻¹ (C≡C); ¹H-NMR (CDCl₃): δ = 1.47 (s, 6H, 2-CH₃), 2.39 (s, 3H, Ph-CH₃), 2.52 (t, 1H, J = 2.4 Hz, HC≡C), 4.66 (d, 2H, J = 2.4 Hz, OCH₂), 5.46 (s, 1H, 3-CH), 6.45 (dd, 1H, J = 2.6 Hz, J = 8.5 Hz, H₆, 6.53 (d, 1H, J = 2.6 Hz, H₈), 6.95 (d, 1H, J = 8.5 Hz, H₅), 7.20 (d, 2H, J = 8.1 Hz, H₃, 5-4-ClPh), and 7.23 (d, 2H, J = 8.2 Hz, H₂, 6-4-ClPh); ¹³C-NMR (CDCl₃): δ = 21.2 (q), 27.6 (q), 55.8 (t), 75.5 (s), 76.3 (s), 78.5 (d), 103.4 (d), 107.5 (d), 116.5 (s), 125.2 (d), 126.4 (d), 128.5 (d), 128.9 (s), 128.9 (d), 134.3 (s), 137.3 (s), 154.6 (s), and 158.5 (s); MS m/z (%): 304 (M⁺, 45), 289 (80), 236 (7), 230 (18), 214 (100), 198 (59), 166 (19), 133 (17), 114 (29), 90 (67), and 76 (51); nal. calcd. for C₂₁H₂₀O₂: C, 82.86; H, 6.62. Found: C, 82.95; H, 6.76.

4-Benzylidene-2,2-dimethyl-7-(prop-2-ynyloxy)chroman (8a) and 4-Benzyl-2,2-dimethyl-7-(prop-2-ynyloxy)-2H-chromene (8b)

These compounds were prepared similarly to 4 using benzylmagnesium bromide. Colorless oil (80%) as a mixture of exo and endo isomers (77:33); R (film): ν 3289 (HC≡C), 2110 cm⁻¹ (C≡C); ¹H-NMR (CDCl₃) 8a
(exo-isomer): $\delta = 1.27_{(Z)}$ and $1.29_{(E)}$ (2s, 6H, 2-CH$_3$), 2.42 (t, 1H, $J = 2.3$ Hz, CH=CH), 2.72 (d, 2H, $J = 1.2$ Hz, 3-CH$_2$), 4.67 (d, 2H, $J = 2.3$ Hz, OCH$_2$), 6.47 (d, 1H, $J = 2.6$ Hz, H$_2$), 6.60 (dd, 1H, $J = 2.6$ Hz, $J = 8.8$ Hz, H$_6$), 7.06 (bs, 1H, Ph=CH = C), 7.29-7.37 (m, 5H, Ar), 7.55 (m, 5H, Ar), and 7.57 $m/z$ 126.2 (d), 126.8 (d), 126.9 (d), 127.1 (d), 127.9 (d), 128.1 (d), 128.4 (d), 128.7 (d), 129.3 (d), 130.1 (s), 137.4 (s), 138.8 (s), 154.4 (s), and 158.7 (s); MS $m/z$ (%): 304 (M$^+$, 51), 303 (100), 288 (74), 222 (14), 199 (19), 177 (32), 166 (22), 114 (28), and 90 (61); nal. calcd. for C$_{21}$H$_{20}$O$_2$: C, 82.86; H, 6.62. Found: C, 82.75; H, 6.69.

4-(2-Chloro-benzylidene)-2,2-dimethyl-7-(prop-2-ynyloxy)chroman (9a) and 4-(2-Chloro-benzyl)-2,2-dimethyl-7-(prop-2-ynyloxy)-2H-chromene (9b)

These compounds were prepared similarly to 4 using 2-chlorobenzylmagnesium chloride. Colorless oil (70%) as a mixture of exo and endo isomers (60:40); R (film): $\nu$ 3295 (HC≡C), 2129 cm$^{-1}$ (C≡C); H-NMR (CDCl$_3$) 9a (exo-isomer): $\delta = 1.27_{(Z)}$ and $1.29_{(E)}$ (2s, 6H, 2-CH$_3$), 2.54 (t, 1H, $J = 2.4$ Hz, CH=CH), 2.57 (d, 2H, $J = 1.3$ Hz, 3-CH$_2$), 4.68 (d, 2H, $J = 2.4$ Hz, OCH$_2$), 6.47 (d, 1H, $J = 2.6$ Hz, H$_2$), 6.61 (dd, 1H, $J = 2.6$ Hz, $J = 8.8$ Hz, H$_6$), 7.04 (bs, 1H, Ph=CH = C), 7.18-7.37 (m, 5H, Ar), and 7.57 $m/z$ 126.2 (d), 126.4 (d), 126.9 (d), 128.1 (d), 128.4 (d), 128.7 (d), 129.2 (d), 130.1 (s), 137.4 (s), 138.8 (s), 154.4 (s), and 158.7 (s); MS $m/z$ (%): 304 (M$^+$, 51), 303 (100), 288 (74), 222 (14), 199 (19), 177 (32), 166 (22), 114 (28), and 90 (61); nal. calcd. for C$_{21}$H$_{20}$O$_2$: C, 82.86; H, 6.62. Found: C, 82.75; H, 6.69.

4-(3-Chloro-benzylidene)-2,2-dimethyl-7-(prop-2-ynyloxy)chroman (10a) and 4-(3-Chloro-benzyl)-2,2-dimethyl-7-(prop-2-ynyloxy)-2H-chromene (10b)

These compounds were prepared similarly to 4 using 3-chlorobenzylmagnesium chloride. Colorless oil (65%) as a mixture of exo and endo isomers (65:35); R (film): $\nu$ 3299 (HC≡C), 2129 cm$^{-1}$ (C≡C); H-NMR (CDCl$_3$) 10a (exo-isomer): $\delta = 1.31_{(E)}$ (s, 6H, 2-CH$_3$), 2.54 (t, 1H, $J = 2.4$ Hz, CH=CH), 2.68 (d, 2H, $J = 1.4$ Hz, 3-CH$_2$), 4.67 (d, 2H, $J = 2.4$ Hz, OCH$_2$), 6.46 (d, 1H, $J = 2.6$ Hz, H$_2$), 6.60 (dd, 1H, $J = 2.6$ Hz, $J = 8.8$ Hz, H$_6$), 6.96 (bs, 1H, Ph=CH = C), 7.15-7.30 (m, 4H, Ar), and 7.54 $m/z$ (d, 1H, $J = 8.8$ Hz, H$_5$). H-NMR (CDCl$_3$) 10b (endo-isomer): $\delta = 1.43$ (s, 6H, 2-CH$_3$), 2.51 (t, 1H, $J = 2.54$ Hz, HC≡C), 3.65 (s, 2H, Ph=CH$_2$), 4.63 (d, 2H, $J = 2.5$ Hz, OCH$_2$), 5.15 (s, 1H, 3-CH), 6.47 (dd, 1H, $J = 2.5$ Hz, $J = 8.4$ Hz, H$_6$), 7.18-7.43 (m, 4H, Ar).
6.49 (d, 1H, J = 2.6 Hz, H₈), 7.00 (d, 1H, J = 8.5 Hz, H₅), and 7.15-7.30 (m, 3H, Ar), 7.54 (d, 1H, J = 8.8 Hz, H₅).; 1H-NMR (CDCl₃)
11a exo-isomer: δ = 1.21 (E) (1s, 6H, 2-CH₃), 2.54 (t, 1H, J = 2.3 Hz, CH=CH), 2.66 (s, 2H, 3-CH₂), 4.67 (d, 2H, J = 2.3 Hz, OCH₃), 6.45 (d, 1H, J = 2.6 Hz, H₈), 6.59 (dd, 1H, J = 2.6 Hz, J = 8.8 Hz, H₅), 6.97 (bs, 1H, 4-Cl-Ph-C=CH = C), 7.21 (d, 2H, J = 8.4 Hz, H₃.₅-C=ClPh), 7.33 (d, 2H, J = 8.4 Hz, H₂.₆-C=ClPh), and 7.54 (E) (d, 1H, J = 8.8 Hz, H₅).; H-NMR (CDCl₃) 11b endo-isomer: δ = 1.41 (s, 6H, 2-CH₃), 2.51 (t, 1H, J = 2.3 Hz, H₃.₅-C=ClPh), 3.64 (s, 2H, 4-Cl-Ph), 7.16 (d, 2H, J = 8.3 Hz, H₂.₆-C=ClPh), 7.26 (d, 2H, J = 8.3 Hz, H₃.₅-C=ClPh), and 7.62 (dd, 1H, J = 2.6 Hz, H₅).; C-NMR (CDCl₃) 11a/11b exo/endo isomers: δ = 26.6 (q), 28.0 (q), 37.2 (t), 55.8 (t), 75.5 (s), 75.6 (s), 75.7 (s), 76.4 (s), 78.4 (d), 78.5 (d), 102.8 (d), 103.2 (d), 107.1 (d), 108.5 (d), 115.3 (s), 116.0 (s), 120.1 (d), 124.3 (d), 125.2 (d), 127.2 (d), 128.4 (d), 128.6 (d), 130.0 (d), 130.6 (d), 130.9 (s), 132.2 (s), 133.7 (s), 154.5 (s), 154.6 (s), 158.5 (s), and 158.9 (s); MS m/z (%): 340 (M⁺ + 2, 27), 339 (M⁺ + 1, 19), 338 (M⁺, 80), 324 (100), 323 (60), 298 (14), 270 (7), 221 (35), 178 (27), 164 (28), 126 (68), 91 (17), and 77 (19).; calcd. for C₂₁H₁₉ClO₂: C, 74.44; H, 5.65.

4-(4-Chlorobenzylidene)-2,2-dimethyl-7-(prop-2-ynyloxy)chroman (11a) and 4-(4-Chlorobenzyl)-2,2-dimethyl-7-(prop-2-ynyloxy)-2H-chromene (11b)

4-(2,3-Dichlorobenzylidene)-2,2-dimethyl-7-(prop-2-ynyloxy)chroman (12a) and 4-(2,3-Dichlorobenzyl)-2,2-dimethyl-7-(prop-2-ynyloxy)-2H-chromene (12b)

These compounds were prepared similarly to 4 using 4-chlorobenzylmagnesium chloride. Colorless oil (80%) as a mixture of exo and endo isomers (50:50): R (film): ν 3291 (HC=HC=CH), 2100 cm⁻¹ (C=C); H-NMR (CDCl₃)
12a exo-isomer: δ = 1.26 (Z) and 1.28 (E) (2s, 6H, 2-CH₃), 2.54 (t, 1H, J = 2.4 Hz, CH=CH), 2.55 (d, 2H, J = 1.4 Hz, 3-CH₂), 4.68 (d, 2H, J = 2.4 Hz, OCH₃), 6.46 (d, 1H, J = 2.6 Hz, H₈), 6.61 (dd, 1H, J = 2.6 Hz, J = 8.8 Hz, H₅), 7.00 (bs, 1H, Ph-C=CH = C), 7.09-7.37 (m, 3H, Ar), 7.56 (Z), and 7.58 (E) (d, 1H, J = 8.8 Hz, H₅).; H-NMR (CDCl₃) 12b endo-isomer: δ = 1.41 (s, 6H, 2-CH₃), 2.50 (t, 1H, J = 2.4 Hz, Ar), 3.80 (s, 2H, Ph-CH₂), 4.64 (d, 2H, J = 2.4 Hz, OCH₂), 5.12 (s, 1H, 3-CH₃), 6.45 (d, 1H, J = 2.6 Hz, J = 8.5 Hz, H₅), 6.48 (d, 1H, J = 2.6 Hz, H₈), 6.95 (d, 1H, J = 8.5 Hz, H₅), and 7.09-7.37 (m, 3H, Ar); C-NMR (CDCl₃) 12a/12b exo/endo isomers: δ = 26.6 (q), 27.9 (q), 35.9 (t), 37.4 (t), 55.8 (t), 75.5 (s), 75.6 (s), 76.4 (s), 78.4 (d), 78.5 (d), 102.8 (d), 103.2 (d), 107.1 (d), 108.5 (d), 115.3 (s), 116.0 (s), 120.1 (d), 124.3 (d), 125.2 (d), 127.2 (d), 128.4 (d), 128.6 (d), 130.0 (d), 130.6 (d), 130.9 (s), 132.2 (s), 133.7 (s), 154.5 (s), 154.6 (s), 158.5 (s), and 158.9 (s); MS m/z (%): 340 (M⁺ + 2, 27), 339 (M⁺ + 1, 19), 338 (M⁺, 80), 324 (100), 323 (60), 298 (14), 270 (7), 221 (35), 178 (27), 164 (28), 126 (68), 91 (17), and 77 (19).; calcd. for C₂₁H₁₉ClO₂: C, 74.44; H, 5.65.

Found: C, 74.57; H, 5.50.
4-(2,4-Dichloro-benzylidene)-2,2-dimethyl-7-(prop-2-ynyloxy)chroman (13a) and 4-(2,4-Dichloro-benzyl)-2,2-dimethyl-7-(prop-2-ynyloxy)-2H-chromene (13b)

These compounds were prepared similarly to 4 using 2,4-dichlorobenzylmagnesium chloride. Colorless oil (77%) as a mixture of exo and endo isomers (53:47); R (film): ν 3288 (HC≡C), 2115 cm⁻¹ (C≡C); H-NMR (CDCl₃) 13a (exo-isomer): δ = 1.28 (E) (s, 6H, 2-CH₃), 2.51 (t, 1H, J = 2.4 Hz, CH≡C), 2.65 (d, 2H, J = 1.5 Hz, 3-CH₂), 4.67 (d, 2H, J = 2.4 Hz, OCH₂), 6.46 (d, 1H, J = 2.6 Hz, H₆), 6.60 (dd, 1H, J = 2.6 Hz, J = 8.8 Hz, H₈), 6.90 (bs, 1H, Ph-CH = C), 7.32-7.42 (m, 3H, Ar), and 7.52 (d, 1H, J = 8.8 Hz, H₅). ¹H-NMR (CDCl₃) 13b (endo-isomer): δ = 1.42 (s, 6H, 2-CH₃), 2.54 (t, 1H, J = 2.4 Hz, HC≡C), 3.62 (s, 2H, Ph-CH₂), 4.63 (d, 2H, J = 2.4 Hz, OCH₂), 5.22 (s, 1H, 3-CH), 6.44 (dd, 1H, J = 2.4 Hz, J = 8.5 Hz, H₆), 6.47 (d, 1H, J = 2.5 Hz, H₈), 6.93 (d, 1H, J = 8.5 Hz, H₅), and 7.05-7.11 (m, 4H, Ar); ¹³C-NMR (CDCl₃) 13a/13b (exo/endo isomers): δ = 26.5 (q), 27.8 (q), 36.8 (t), 37.0 (t), 55.6 (t), 75.3 (s), 75.6 (s), 75.7 (s), 76.2 (s), 78.3 (d), 102.7 (d), 103.2 (d), 107.0 (d), 108.4 (d), 114.6 (s), 115.5 (s), 118.7 (d), 121.4 (d), 125.1 (d), 125.5 (d), 127.4 (s), 128.0 (d), 129.4 (s), 130.0 (d), 130.1 (s), 130.2 (d), 130.4 (d), 130.8 (d), 130.9 (s), 131.0 (s), 135.4 (s), 150.4 (s), 150.6 (s), 150.9 (s); MS m/z (%): 376 (M⁺ + 4, 3), 375 (M⁺ + 3, 6), 374 (M⁺ + 2, 18), 373 (M⁺ + 1, 6), 372 (M⁺, 28), 361 (45), 358 (100), 317 (9), 256 (13), 190 (9), 160 (18), 116 (9), and 70 (9); nal. calcd. for C₂₁H₁₈Cl₂O₂: C, 67.57; H, 4.86. Found: C, 67.70; H, 4.90.

4-(2,6-Dichloro-benzylidene)-2,2-dimethyl-7-(prop-2-ynyloxy)chroman (14a) and 4-(2,6-Dichloro-benzyl)-2,2-dimethyl-7-(prop-2-ynyloxy)-2H-chromene (14b)

These compounds were prepared similarly to 4 using 2,6-dichlorobenzylmagnesium chloride. Colorless oil (76%) as a mixture of exo and endo isomers (27:73); R (film): ν 3297 (HC≡C), 2130 cm⁻¹ (C≡C); H-NMR (CDCl₃) 14a (exo-isomer): δ = 1.28 (1s, 6H, 2-CH₃), 2.53 (t, 1H, J = 2.4 Hz, CH≡C), 2.67 (s, 2H, J = 1.3 Hz, 3-CH₂), 4.67 (d, 2H, J = 2.4 Hz, OCH₂), 6.48 (d, 1H, J = 2.6 Hz, H₆), 6.60 (dd, 1H, J = 2.6 Hz, J = 8.8 Hz, H₈), 6.71 (bs, 1H, 4-Cl-Ph-CH = C), 7.20-7.35 (m, 3H, Ar), and 7.69 (d, 1H, J = 8.8 Hz, H₅). ¹H-NMR (CDCl₃) 14b (endo-isomer): δ = 1.30 (s, 6H, 2-CH₃), 2.53 (t, 1H, J = 2.4 Hz, HC≡C), 3.95 (s, 2H, 4-Cl-Ph-CH₂), 4.68 (d, 2H, J = 2.4 Hz, OCH₂), 5.21 (s, 1H, 3-CH), 6.46 (dd, 1H, J = 2.6 Hz, J = 8.5 Hz, H₆), 6.48 (d, 1H, J = 2.6 Hz, H₈), 6.97 (d, 1H, J = 8.5 Hz, H₅), and 7.20-7.35 (m, 3H, Ar); ¹³C-NMR (CDCl₃) 14a/14b (exo/endo isomers): δ = 26.6 (q), 27.8 (q), 32.3 (t), 38.0 (t), 55.7 (t), 75.5 (s), 75.6 (s), 76.5 (s), 78.5 (d), 102.7 (d), 103.2 (d), 107.0 (d), 108.3 (d), 114.5 (s), 115.9 (d), 116.3 (s), 123.3 (d), 123.7 (d), 125.5 (d), 126.8 (s), 127.8 (d), 128.1 (d), 128.3 (d), 134.7 (s), 135.6 (s), 136.4 (s), 154.3 (s), 154.7 (s), 158.4 (s), 158.5 (s), and 159.2 (s); MS m/z (%): 376 (M⁺ + 4, 3), 375 (M⁺ + 3, 6), 374 (M⁺ + 2, 18), 373 (M⁺ + 1, 6), 372 (M⁺, 28), 361 (45), 358 (100), 317 (9), 256 (13), 190 (9), 160 (18), 116 (9), and 70 (9); nal. calcd. for C₂₁H₁₈Cl₂O₂: C, 67.57; H, 4.86. Found: C, 67.60; H, 4.75.

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and 159.1 (s); MS m/z (%): 376 (M$^+$ + 4, 4), 375 (M$^+$ + 3, 5), 374 (M$^+$ + 2, 22), 373 (M$^+$ + 1, 6), 372 (M$^+$, 35), 358 (100), 319 (9), 303.1 (5), 256.1 (12), 219 (9), 160 (11), 116 (5), 90 (6), and 76 (6).; nal. calcd. for C$_{21}$H$_{18}$Cl$_2$O$_2$: C, 67.57; H, 4.86. Found: C, 67.64; H, 4.73.

4-(4-Fluoro-benzylidene)-2,2-dimethyl-7-(prop-2-ynyloxy)chroman (15a) and 4-(4-Fluoro-benzyl)-2,2-dimethyl-7-(prop-2-ynyloxy)-2H-chromene (15b)

These compounds were prepared similarly to 4 using 4-fluorobenzylmagnesium chloride. Colorless oil (75%) as a mixture of exo and endo isomers (47:53).; R (film): $\nu$ 3294 (HC≡C), 2120 cm$^{-1}$ (C≡C).; H-NMR (CDCl$_3$) 15a (exo-isomer): $\delta$ = 1.27 (Z) and 1.29 (E) (2s, 6H, 2-CH$_3$), 2.54 (t, 1H, $J$ = 2.4 Hz, CH≡C), 2.67 (s, 2H, $J$ = 1.3 Hz, 3-CH$_2$), 4.67 (d, 2H, $J$ = 2.4 Hz, OCH$_2$), 6.48 (d, 1H, $J$ = 2.6 Hz, H$_8$), 6.60 (dd, 1H, $J$ = 2.6 Hz, $J$ = 8.8 Hz, H$_6$), 6.96 (bs, 1H, 4-Cl-Ph-CH = C), 6.98-7.26 (m, 3H, Ar), 7.53 (Z), and 7.55 (E) (d, 1H, $J$ = 8.8 Hz, H$_5$).; H-NMR (CDCl$_3$) 15b (endo-isomer): $\delta$ = 1.40 (s, 6H, 2-CH$_3$), 2.51 (t, 1H, $J$ = 2.3 Hz, HCC≡C), 3.65 (s, 2H, 4-Cl-Ph-CH$_2$), 4.63 (d, 2H, $J$ = 2.4 Hz, OCH$_2$), 5.20 (s, 1H, 3-CH), 6.44 (dd, 1H, $J$ = 2.6 Hz, $J$ = 8.5 Hz, H$_6$), 6.47 (d, 1H, $J$ = 2.6 Hz, H$_8$), 6.97 (d, 1H, $J$ = 8.5 Hz, H$_5$), and 6.98-7.26 (m, 3H, Ar).; C-NMR (CDCl$_3$) 15a/15b (exo/endo isomers): $\delta$ = 26.6 (q), 28.0 (q), 36.9 (t), 37.1 (t), 55.7 (t), 55.8 (t), 75.4 (s), 75.5 (s), 75.6 (s), 76.4 (s), 78.4 (d), 102.8 (d), 103.2 (d), 106.9 (d), 107.0 (d), 108.4 (d), 114.9 (s), 115.1 (s), 115.2 (s), 116.2 (s), 120.2 (d), 124.3 (d), 125.1 (d), 127.0 (d), 129.8 (s), 130.0 (d), 130.1 (d), 130.3 (s), 130.8 (d), 130.9 (d), 133.4 (s), 134.4 (s), 154.4 (s), 154.5 (s), 155.4 (s), 158.4 (s), 160.4 (s), 160.5 (s), and 162.4 (s); MS m/z (%): 323 (M$^+$ + 1, 19), 322 (M$^+$, 82), 307.2 (100), 267.0 (10), 195.7 (13), 145.0 (5), 109 (41), and 69 (9).; nal. calcd. for C$_{21}$H$_{19}$FO$_2$: C, 78.24; H, 5.94. Found: C, 78.41; H, 5.76.

Biological Activity

The L. major strain used in the present study was the vaccine strain (MRHO/IR/75/ER) and was obtained from the Pasteur Institute, Tehran, Iran. Infectivity of the parasites was maintained by regular passage in susceptible BALB/c mice. The promastigote form of the parasite was grown in NNN-blood agar medium at 25°C. The stationary parasite inoculation was $2 \times 10^6$ cells/mL. For the experiments described herein stationary phase promastigotes were washed with phosphate-buffered saline (PBS) and recultured in RPMI 1640 medium (pH $\sim$ 7.2) at $2 \times 10^6$ cells/mL, supplemented with 10% of heat-inactivated fetal bovine serum (FBS), 2 mM of glutamine, 100 U/mL of penicillin, and 100 $\mu$g/mL of streptomycin.$^{14,15}$

Determination of L. major Growth Kinetics

First, the growth curve of the parasite was determined by daily enumeration using a Neubauer’s chamber and light microscopy. Then, $2 \times 10^6$/mL of log phase promastigotes were inoculated into 15 mL of fresh RPMI 1640 medium in T25 culture flasks; the current antileishmanial drug glucantime was the reference. Assessment of growth by cell enumeration (Neubauer Chamber) was determined at 24-h intervals for 8 days. The values obtained were used to determine the relative growth rate and the results were expressed as the mean values of at least 3 experiments.
In Vitro *L. major* Promastigote Culture and Compound Toxicity Assays

In vitro growth inhibition of the chromenes was evaluated by direct enumeration under light microscopy. Briefly, promastigotes in the logarithmic growth phase were incubated at an average of $2 \times 10^6$ cells/mL in RPMI 1640 medium supplemented with 10% FBS, as described above, in the presence of chromene compounds dissolved in dimethylsulphoxide (DMSO), and were incubated for 24 h, 48 h, and 72 h at 25 °C. Promastigotes in the control groups were incubated with DMSO alone, or with the antimonial drug glucantime. Finally, the number of promastigotes was recorded for at least 3 independent experiments and the results are expressed as the mean percentage reduction of parasites in comparison with the untreated controls. The percentage of growth inhibition caused by the synthesized compounds in a concentration of 10 μg/mL was determined by counting the number of treated promastigotes remaining alive in the cultures (1-cell number of drug-treated culture/cell number of control culture) × 100), and by analyzing the growth inhibition of the parasite as described above. The results are shown in Figure 3 and the Table.

Statistical Analysis

Student’s t-test was used to compare the susceptibility of the logarithmic phase parasites to the compounds. The level of significance was a P value > 0.05.

*L. major* Growth Curve

A general procedure was used in the current study to identify 3 different growth phases of the promastigotes. The growth kinetics for a period of 8 days is shown in Figure 2. The logarithmic phase of the promastigotes lasted until day 3; thereafter, the parasites gradually entered the stationary phase. The maximum cell concentration of $1.1 \times 10^7$/mL was achieved after 96 h. In addition, we assessed the growth curve of the promastigotes treated with glucantime, which showed a gradually reduction during 5 days. Determination of growth inhibition due to the drug was made when the parasites were still in the log phase, prior to entering the stationary phase.

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References